Spike Trimer (S1+S2) (B.1.1.7 Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit

#78175
96 reactions

Description

The COVID-19 pandemic is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The Spike glycoprotein is expressed on the surface of the virus as a trimer. Each Spike protein consists of two subunits, S1 and S2, and the S1 subunit has a receptor binding domain (RBD) which recognizes and attaches to the ACE2 receptor found on the surface of type I and II pneumocytes, endothelial cells, and ciliated bronchial epithelial cells. The SARS-CoV-2 Variant B.1.1.7 was originally discovered in the United Kingdom. It contains mutations N501Y, A570D, D614G, P681H, T716I, S982A, D1118; deletions: 21765:6 (69-70HV), 21991:3 (144Y). Drugs targeting the interaction between the Spike protein of SARS-CoV-2 and ACE2 may offer some protection against the viral infection. The SARS-CoV-2 Spike Trimer (S1+S2) (B.1.1.7 Variant):ACE2 Inhibitor Screening Colorimetric Assay Kit includes the UK Variant Spike protein in its native trimeric conformation to provide a more physiologically relevant screen for inhibitors of the Spike S1:ACE2 interaction.

The SARS-CoV-2 Spike Trimer (S1+S2) (B.1.1.7 Variant):ACE2 Inhibitor Screening Colorimetric Assay Kit is designed for screening and profiling inhibitors or neutralizing antibodies of this interaction. This kit comes in a convenient 96-well format, with Biotinylated-ACE2, purified Spike Trimer (S1+S2) (B.1.1.7 Variant) protein (His-tagged), Streptavidin-HRP, and assay buffers for 100 binding reactions. The key to this kit is that the SARS-CoV-2 Spike Trimer (S1+S2) (B.1.1.7 Variant) protein provides a more biologically relevant model than monomeric Spike RBD protein for the investigation of SARS-CoV-2/host cell interaction. Only a few simple steps on a microtiter plate are required for the assay. First, SARS-CoV-2 Spike Trimer (S1+S2) (B.1.1.7 Variant) is coated on a 96-well plate overnight. Next, the proteins are blocked and pre-incubated with the inhibitor or neutralizing antibody. Upon subsequent incubation with Biotin-ACE2, the plate is treated with Streptavidin-HRP followed by addition of a colorimetric HRP substrate to produce color, which then can be quenched and measured using a UV/Vis microplate reader.

Applications

- Useful for screening for inhibitors of ACE2 binding to trimeric SARS-CoV-2 Spike Trimer (S1+S2) (B.1.1.7 Variant)

Supplied Materials

Catalog #	Name	Amount	Storage
510334	Spike Trimer (S1+S2) (B.1.1.7 Variant), His-Tag (SARS-CoV-2)	5 µg	-80°C
100665	ACE2, His-Avi-Tag, Biotin labeled	5 µg	-80°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79742	Streptavidin-HRP	5 µl	+4°C
	Colorimetric HRP substrate	10 ml	+4°C
79964	Transparent 96-well microplate	1	Room Temp

Materials Required but Not Supplied

PBS (Phosphate buffered saline)
1N HCl (aqueous)
Rotating or rocker platform
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*

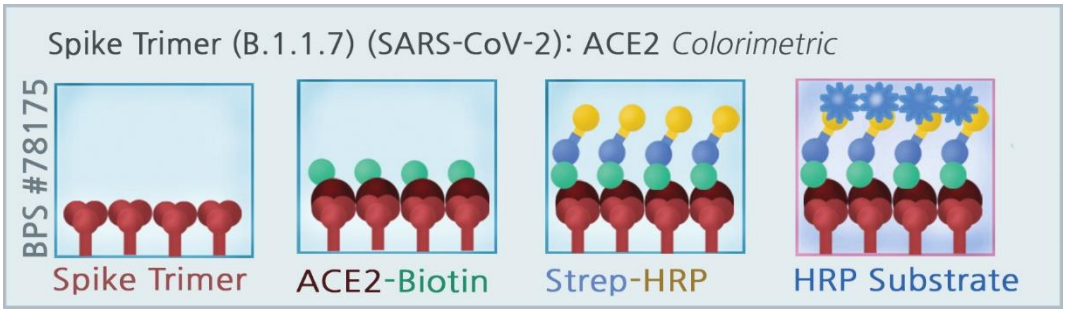
Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle



Assay Protocol

All samples and controls should be tested in duplicate. We recommend preincubating the enzyme with inhibitor.

Day 1-Coating the plate with SARS-CoV-2 Spike Trimer protein:

1) Thaw **Spike Trimer (S1+S2) (B.1.1.7 Variant) protein** on ice. Upon first thaw, briefly spin tube to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining in aliquots at -80°C. Note: **Spike protein** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **Spike Trimer (S1+S2) (B.1.1.7 Variant) protein** to 1 µg/ml in PBS.
3) Add 50 µl of diluted **Spike Trimer (S1+S2) (B.1.1.7 Variant) protein** solution to each well and incubate at 4°C overnight.

Day 2-Blocking:

1) Prepare **1x Immuno Buffer** by diluting **3x Immuno Buffer** in distilled water (one portion of **3x Immuno Buffer** is added to two portions of distilled water).
2) After the overnight coating, discard the solution and wash the plate three times with 100 µl of **1x Immuno Buffer 1** per well. Tap plate onto clean paper towels to remove liquid.
3) Block wells by adding 100 µl **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature

with slow shaking. Remove the blocking solution and wash three times with 100 µl of **1x Immuno Buffer 1**. Tap the plate onto clean paper towels to remove liquid.

Day 2-Blocking and binding assay:
****Note there are two methods for steps 4-8 depending on your inhibitor****
If testing antibody as inhibitor, follow Steps 4-8 below:

4) Prepare dilutions of neutralizing anti-Spike antibody in **Blocking Buffer 2** desired concentration (it is recommended to use serial dilutions of the neutralizing antibody). Prepare enough for 25 uL per well.

5) Add 25 µl of the diluted antibody to the "Test Inhibitor" wells. To the wells labeled "Blank" and "Positive Control", add 25 µl **Blocking Buffer 2**. Incubate the plate for 1 hour at room temperature with slow rotation.
6) Thaw the **Biotin-ACE2** on ice, and dilute it to 1.5 ng/µl in **Blocking Buffer 2**. Prepare only the amount required for the assay; store remaining **Biotin-ACE2** undiluted at -80°C. Note: **Biotin-ACE2** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
7) After 1 hour incubation of the antibody, add 25 µl of diluted **Biotin-ACE2** to the wells labeled "Test Inhibitor" and "Positive Control". Add 25 µl **Blocking Buffer 2** to the wells labeled "Blank". At this step, there should be a total of 50 uL volume in each well. Incubate the plate at room temperature for another 1 hour with slow rotation.

Component	Blank	Positive Control	Test Inhibitor
Blocking Buffer 2	50 µl	25 µl	-
Test antibody	-	-	25 µl
ACE2-Biotin (1.5 ng/µl)	-	25 µl	25 µl
Total	50 µl	50 µl	50 µl

8) After 1 hour, discard the solution and wash the plate three times with 100 µl of 1x Immuno Buffer 1. Tap the plate onto clean paper towels to remove liquid.

If testing small molecule inhibitor, follow steps 4-8 below:

4) Prepare the test inhibitor in DMSO (or distilled water if soluble), and further dilute it in distilled water at 10X testing concentration. (*e.g.* To test a compound at 10 µM, prepare the inhibitor in DMSO at 1 mM. Then make a 10-fold dilution distilled water to create a 100 µM solution in 10% DMSO(aq)).
5) Add 5 µl to each well labeled "Test Inhibitor". To the "Positive Control" and "Blank" wells, add 5 µl of the same solution without inhibitor (e.g. 10% DMSO(aq) solution) so that all wells contain the same amount of DMSO. *Caution! – It is highly recommended that the final DMSO concentration should not exceed 1%. Organic solvents other than DMSO are not validated in this assay, so use of these solvents must be optimized by the user*.
6) Thaw the **Biotin-ACE2** on ice, and dilute it in **Blocking Buffer 2** at 1.5 ng/µl. Prepare only the amount required for the assay; store remaining **Biotin-ACE2** undiluted at -80°C. Note: **Biotin-ACE2** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
7) Add 20 µl of **Blocking Buffer 2** to the wells labeled "Blank". Add 20 µl of diluted **Biotin-ACE2** to the wells labeled "Test Inhibitor" and "Positive Control". Incubate the plate at room temperature for 1 hour with slow rotation.

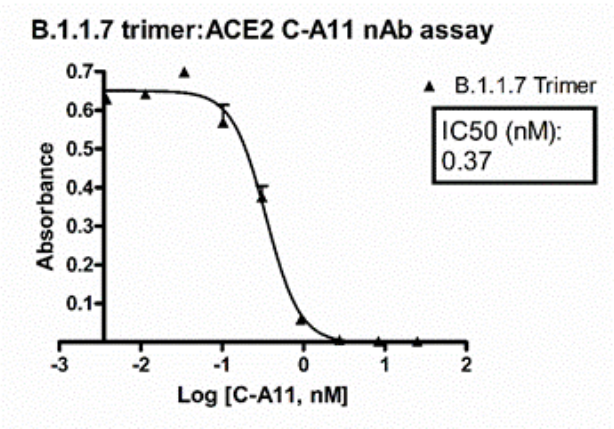
Component	Blank	Positive Control	Test Inhibitor
Blocking Buffer 2	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Inhibitor solution (no inhibitor) – usually 10% DMSO(aq)	5 µl	5 µl	-
ACE2-Biotin (1.5 ng/µl)	-	20 µl	20 µl
Total	50 µl	50 µl	50 µl

8) After 1 hour, discard the solution and wash the plate three times with 100 uL of Blocking Buffer 2. Tap the plate onto clean paper towels to remove liquid.

Day 2-Detection:

9) Dilute **Streptavidin-HRP** 1000-fold with the **Blocking Buffer 2**, enough for 50 ul per well.
10) Add 50 µl of the **diluted Streptavidin-HRP** to each well and incubate the plate for 30 min at room temperature with slow rotating.
11) After 30 minutes, discard the solution and wash the plate three times with 100 µl of **1x Immuno Buffer 1**. Tap the plate onto clean paper towel to remove liquid.
12) Prepare enough 1M HCl (aqueous-stop solution) for 100 ul per well. *Note: alternatively, 2N H_2SO_4 or other compatible acidic solutions can be substituted.*
13) Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until blue color is developed in the 'Positive Control' wells. This usually takes a 1-5 minutes. The optimal incubation time may vary, and should be determined empirically by the user. It is recommended that the reaction be stopped when the 'Positive Control' well is lower than ~ 1.0 absorbance at 450 nm (preferably ~ 0.6).
14) Once blue color is developed in the 'Positive Control' well, add 100 µl of HCL stop solution prepared above. The blue color should now be yellow.
15) Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader.

Example Results



Inhibition of ACE2:SARS-CoV-2 Spike Trimer (B.1.1.7 variant) binding by an anti-SARS-CoV-2 Spike neutralizing antibody. C-A11, an anti-SARS-CoV-2 Spike antibody (BPS Bioscience, #101024) was evaluated using the SARS-CoV-2 Spike Trimer (B.1.1.7 variant):ACE2 Inhibitor Screening Colorimetric Assay Kit. The antibody was serially diluted from 25 to 0 nM in 3-fold and tested following the assay kit protocol. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General considerations

Plates and Instruments: A plate reader capable of Alpha technology detection is required. We recommend using PerkinElmer 384-Optiplate #6007290.

"Blank" Control: The "Blank" control is important to determine the background luminescence in the assay. We recommend doing these in duplicate.

"Positive Control": The "Positive Control" is the maximum signal determined by the addition of a system with DMSO, in our case the "10% DMSO in water (Inhibitor buffer)."

Trouble Shooting Guide

Problem	Possible Causes	Recommended Solutions
Luminescence signal of positive control reaction is weak	Spike and ACE2-Biotin have lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh proteins. Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Colorimetric HRP substrate was not incubated long enough	Increase the amount of time that the colorimetric HRP substrate is incubated in the wells. Avoid azides.
Alpha-counts signal is erratic or varies widely among wells	Inaccurate pipetting/ technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Signal is out of range of detection (too high)	Decrease the amount of time that the colorimetric HRP substrate is incubated in the wells
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of proteins to create a standard curve

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Spike Trimer (S1+S2) (B.1.1.7 Variant), His-Tag (SARS-CoV-2)	510334	100 µg/1 mg
ACE2, His-Avi-Tag, Biotin-labeled HiP™	100665	20 µg/50 µg
Spike Trimer (S1+S2), His-tag (SARS-CoV)	100789	100 µg/500 µg
Spike Trimer (S1+S2), His-tag (HCoV-NL63)	100788	100 µg/500 µg
Spike Trimer (S1+S2), His-tag (SARS-CoV-2)	100728	100 µg/1 mg
SARS-CoV-2 Spike:ACE2 Inhibitor Screening Assay Kit	79931	96 reactions
ACE2:SARS-CoV-2 Spike Inhibitor Screening Assay Kit	79936	96 reactions
ACE2:SARS-CoV-2 Spike S1-Biotin Inhibitor Screening Assay Kit	79945	96 reactions
SARS-CoV-2 Spike S1-Biotin:ACE2 TR-FRET Assay Kit	79949	96 reactions
Spike S1, Fc Fusion, Avi-tag (SARS-CoV-2)	100678	100 µg/1 mg
Spike S1, Fc fusion, Avi-tag, Biotin-Labeled (SARS-CoV-2)	100679	25 µg/50 µg
Spike S1 RBD, His-tag (SARS-CoV-2)	100687	50 µg/100 µg
Spike S1, Fc fusion (SARS-CoV-2)	100688	20 µg/50 µg
Spike S1 RBD, Fc fusion (SARS-CoV-2)	100699	50 µg/100 µg
ACE2 Inhibitor Screening Assay Kit	79923	96 reactions
ACE2, His-tag	11003	20 µg/100 µg